Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the in-house investigations of **Calcium Gluconate** in the developing chicken embryo 5/1/74

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GRM107

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: May 1, 1974

THRU : Dr. Leo Friedman, Director
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph.D.
Reproductive Physiology Branch, HFF-157

M. Jacqueline Verrett

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Calcium Gluconate in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Calcium Cluconate

Protocol:

Calcium Gluconate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Column 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5, and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Calcium gluconate showed no toxicity when injected via the air cell at either 0 or 96 hours. Injection into the yolk showed moderate toxicity and calcium gluconate was 100% lethal at 50 mg/kg when injected at 96 hours. Under all four test conditions the slope of the regression line was not significantly different from zero ($p=0.05$).

There were few serious anomalies observed for all four modes of treatment and the incidences for the individual dose levels were not high when compared to the solvent-treated controls. Air cell treatment at 0 hours produced three serious abnormalities: 25.0 mg/kg, one exencephaly and one buphthalmia, and at 5.0 mg/kg, one bird with a cataract. The solvent controls also had one exencephaly. Air cell treatment at 96 hours also had three abnormal birds: 25.0 mg/kg one exencephaly and one ablepharia, and 1.250 mg/kg, one microphthalmia. Yolk treatment at 0 hours caused no serious abnormalities. Yolk injection at 96 hours had six birds as follows: 25.0 mg/kg, exencephaly, two birds, and one with microphthalmia; 12.50 mg/kg, one bird with micromelia; and 2.50 mg/kg, two birds, one with celosomia, and one with ablepharia, cleft palate, and dysgnathia. There were three abnormals in the solvent-treated controls; one exophthalmia, one coloboma, and one microphthalmia. The untreated controls had two serious abnormalities, brachygnathia and microphthalmia.

Under the test conditions employed, calcium gluconate showed only moderate embryotoxicity and was not teratogenic between 1.250 and 50 mg/kg.

1. Calcium Gluconate, Lot #72993, Pfizer Chemical Co.

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006.

4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Table 1

Calcium Gluconate

Air Cell at 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
2.50	50.00	85	31.76	1.17	0.00
1.250	25.00	85	32.94	7.05	3.52
0.500	10.00	85	32.94	3.52	1.17
0.250	5.00	85	32.94	2.35	1.17
0.1250	2.50	85	22.35	2.35	1.17
Water		120	30.83	1.66	0.83
Controls		418	20.81	1.91	1.43

**Slope not significantly different from zero ($p=0.05$)

Table 2

Calcium Gluconate

Air Cell at 96 Hours

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
2.50	50.00	20	35.00	0.00	0.00
1.250	25.00	130	40.76*	3.07	0.76
0.6250	12.50	110	39.09*	3.63	3.63
0.500	10.00	20	30.00	0.00	0.00
0.250	5.00	130	37.69*	0.76	0.00
0.1250	2.50	130	38.46*	0.76	0.76
0.06250	1.250	110	26.36	1.81	0.90
Water		145	21.37	1.37	0.00
Controls		418	20.81	1.91	1.43

**Slope is not significantly different from zero ($p=0.05$)

* Significantly different from solvent ($p<0.05$)

Table 3

Calcium Gluconate

Yolk at 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
2.50	50.00	80	87.50*	0.00	0.00
1.250	25.00	80	88.75*	2.50	0.00
0.500	10.00	80	92.50*	1.25	1.25
0.250	5.00	80	77.50*	2.50	0.00
0.1250	2.50	79	77.21*	1.26	0.00
Water		115	44.34	0.86	0.00
Controls		418	20.81	1.91	1.43

**Slope is not significantly different from zero ($p=0.05$)

* Significantly different from solvent ($p \leq 0.05$)

Table 4

Calcium Gluconate

Yolk at 96 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
2.50	50.00	25	100.00*	4.00	0.00
1.250	25.00	140	71.42*	6.42	2.85
0.6250	12.50	114	61.40*	3.50	1.75
0.500	10.00	25	96.00*	0.00	0.00
0.250	5.00	140	74.28*	4.28	2.14
0.1250	2.50	140	61.42*	7.14	3.57
0.06250	1.250	114	56.14*	2.63	0.00
Water		145	28.96	4.13	2.06
Controls		418	20.81	1.91	1.43

**Slope is not significantly different from zero ($p=0.05$)

* Significantly different from solvent ($p \leq 0.05$